Microfluidic Detection of *Escherichia coli* in Macro Water Distribution System

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• Motivation

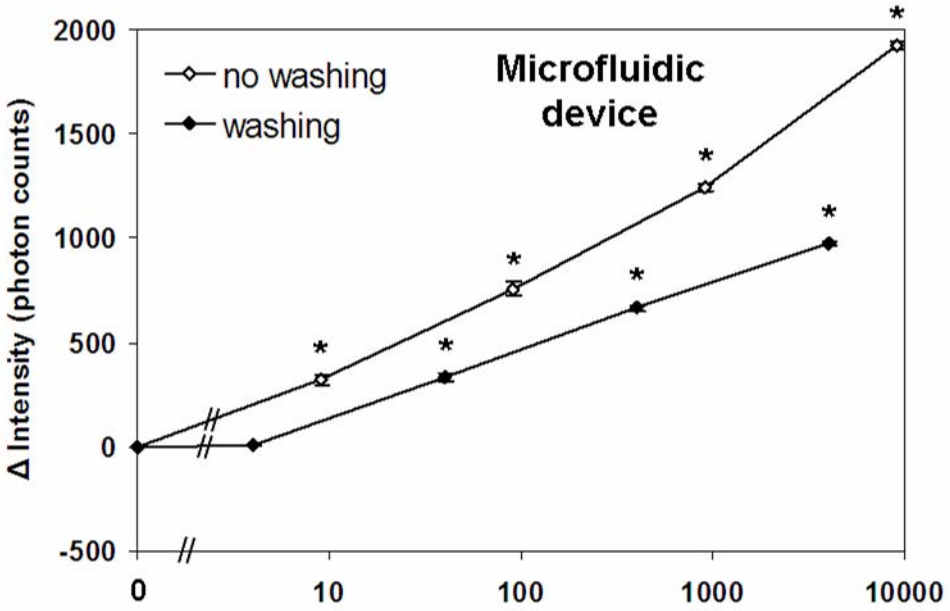
- **Concern has recently been raised about vulnerability of water supply system to terrorist attack**
  - Pathogenic contamination in drinking and irrigation water threatens the environment and public health

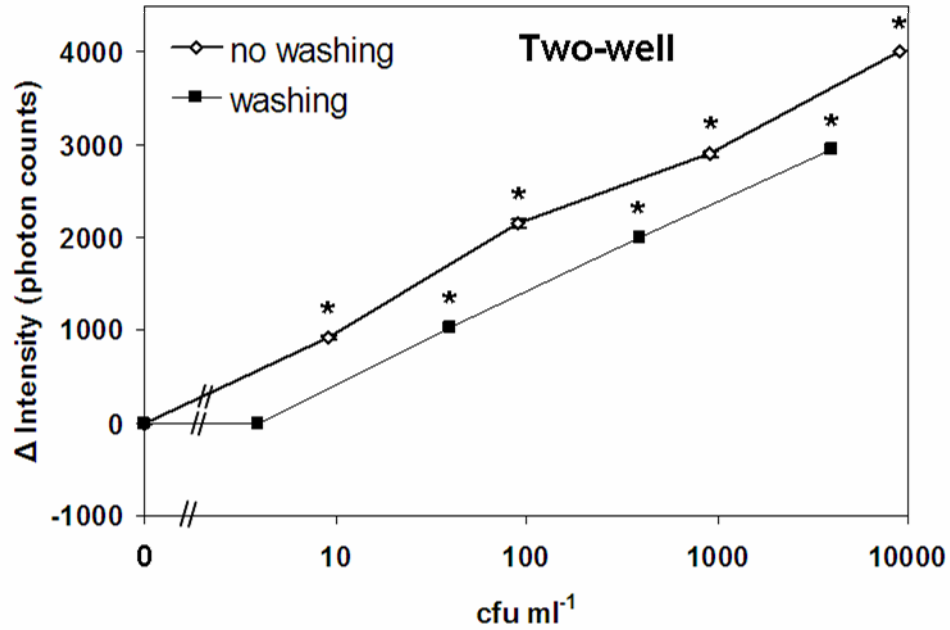
- **Accurate and real-time risk assessment of biological agent is a critical issue**
  - Developing quantitative sensor to monitor water quality
  - Improving accuracy in predicting transport phenomena of the biological agents in water distribution system

Previous studies

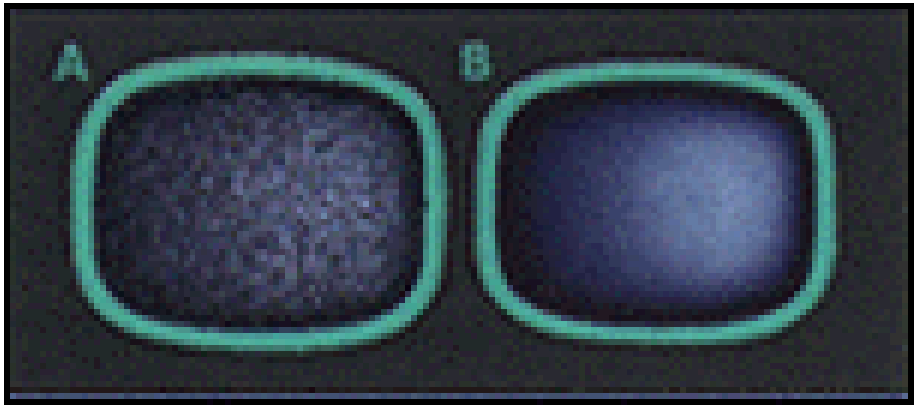
• Detecting waterborne pathogens in a microfluidic device

- **More sensitive and faster detection than PCR or ELISA**
- **Lin et al.,** *Biomed. Microdev.* **6: 125 (2004)**
  - Immunoassay for *E. coli* O157:H7 using colorimetric quantification in a PDMS microfluidic device (detection limit: 10 ng or 3×10^4 cells)
- **Li and Su,** *J. Rapid Meth. Autom. Microbiol.* **14: 96 (2006)**
  - Capturing *E. coli* O157:H7 with the antibodies immobilized in a microfluidic device, followed by detection with a UV/vis spectrometer
- **Han et al.,** *Biosens. Bioelectron.* **doi:10.1016/j.bios.2007.11.013 (2008)**
  - Real-time detection of viable *E. coli* only through light scattering of latex immunoagglutination in a microfluidic device (detection limit: 4 cfu per device)


Microfluidic device
no washing
washing
Δ Intensity (photon counts)
2000
1500
1000
500
0
-500
0
10
100
1000
10000

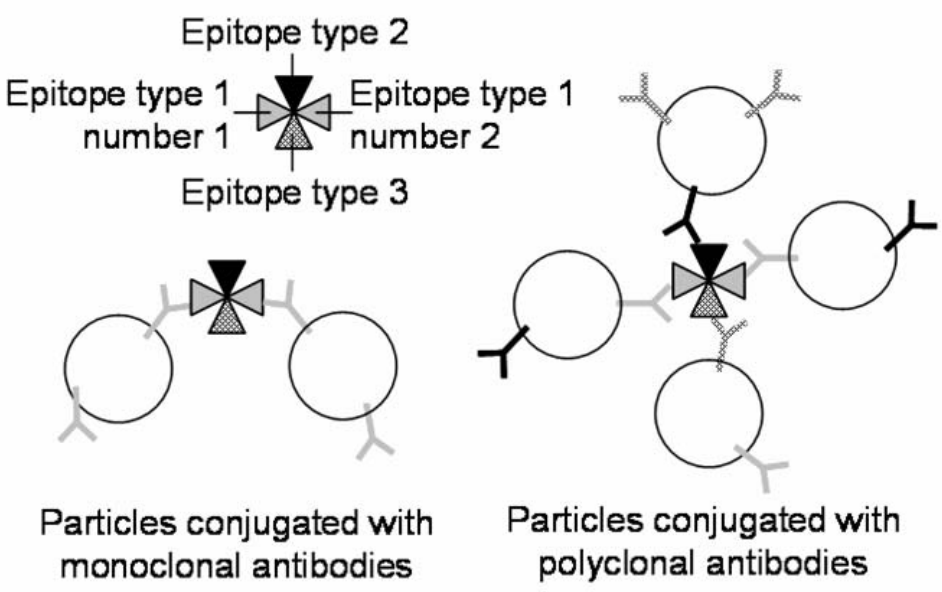

Two-well
no washing
washing
Δ Intensity (photon counts)
4000
3000
2000
1000
0
-1000
0
10
100
1000
10000
cfu ml-1

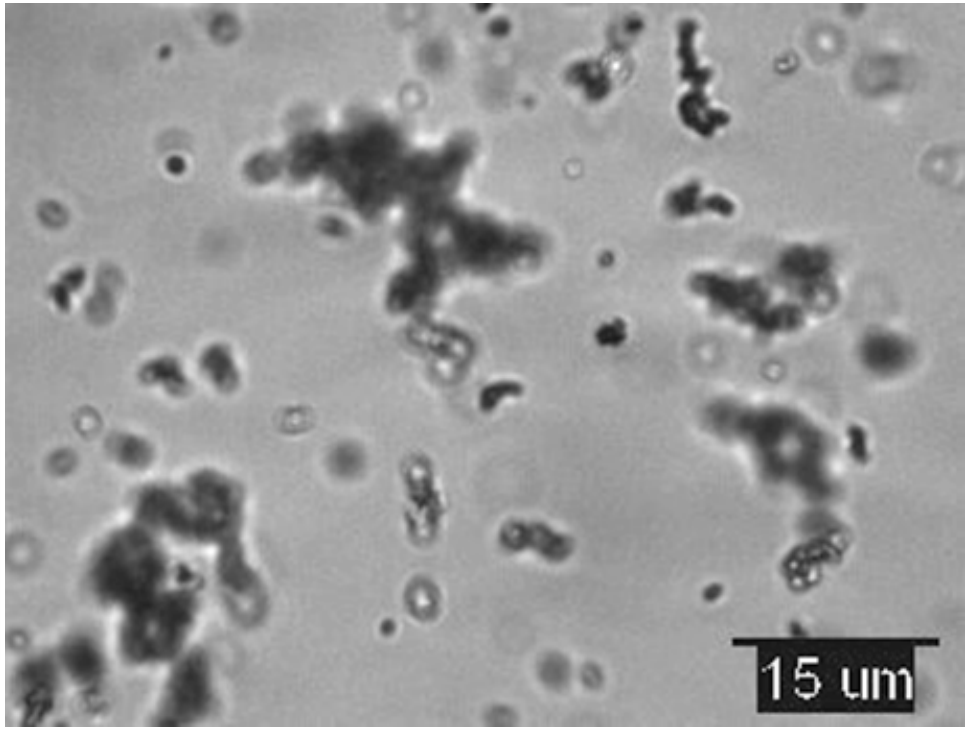
• What is latex agglutination test (LAT)?



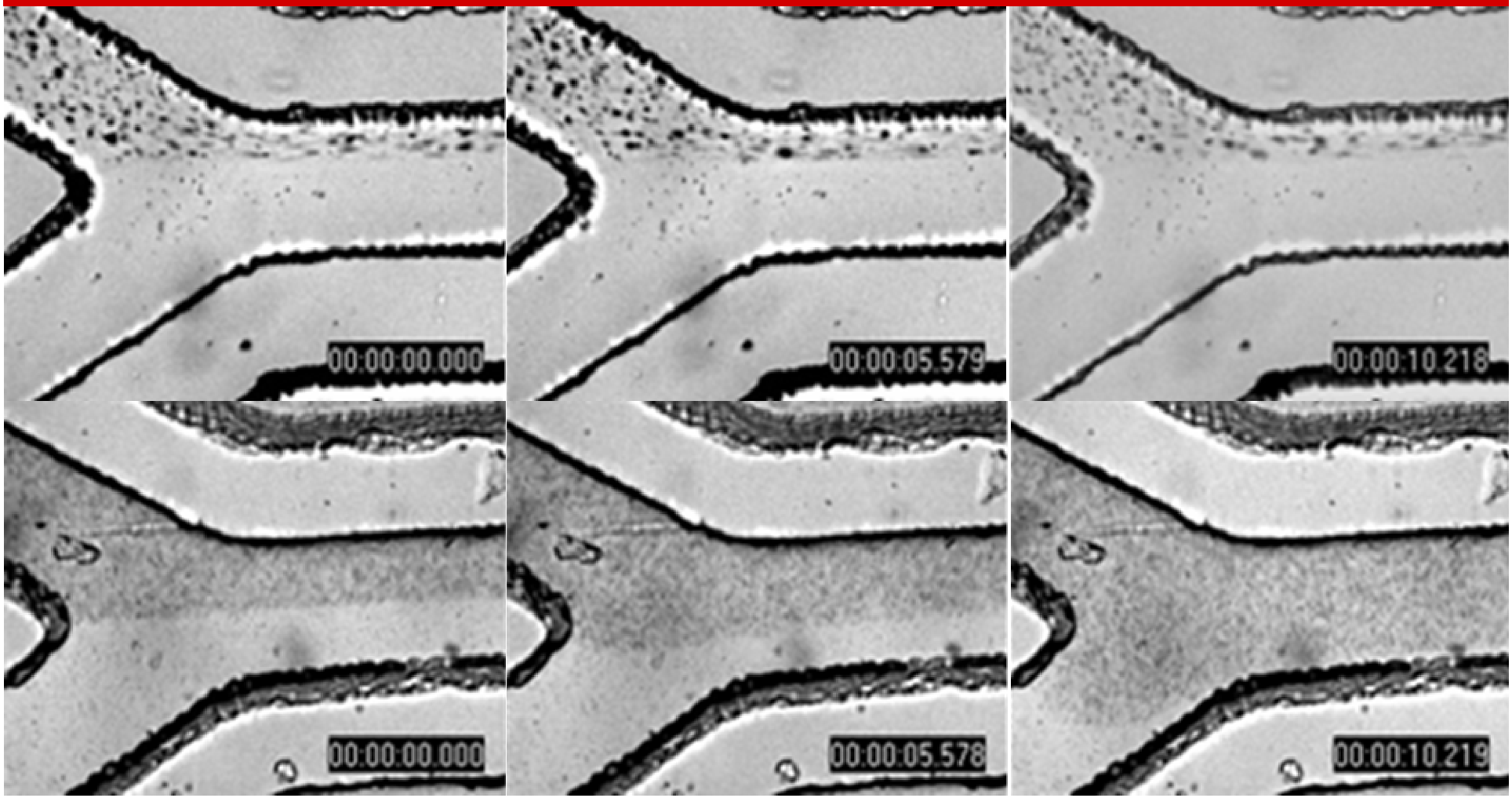
- Polymer microparticles, or latex, conjugated with antibodies, are mixed to target specimen.
- Existence of complementary antigens in the specimen results in connecting two or more particles, known as agglutination.
- This agglutination can visually identified.

• Particle immunoagglutination in microfluidics





- **Particle immunoagglutination in microfluidics**



Han et al. *Anal. Chim. Acta* **584: 252 (2007)**

• Predicting transport phenomena of a pipe flow

- **Molecular transport in a pipe flow has been extensively studied since 1950s**
- **Taylor,** *Proc. R. Soc. London A* **219: 186 (1953); 225: 473 (1954)**
  - Analyzed the dispersion of salt injected into a capillary tube.
  - The axial dispersion model was valid when dimensionless time is much higher than 0.7 ($T = 4\ D\ t/d^2$) and Peclet number is higher than 500 ($Pe = a\ u_0/D$)
  - D = molecular diffusion coefficient, t = elapsed time, d = inner diameter of a pipe, a = radius of a pipe, u_0 = maximum flow velocity
  - Concentration profile of salt tracer = symmetric in laminar flow regarding axial convection

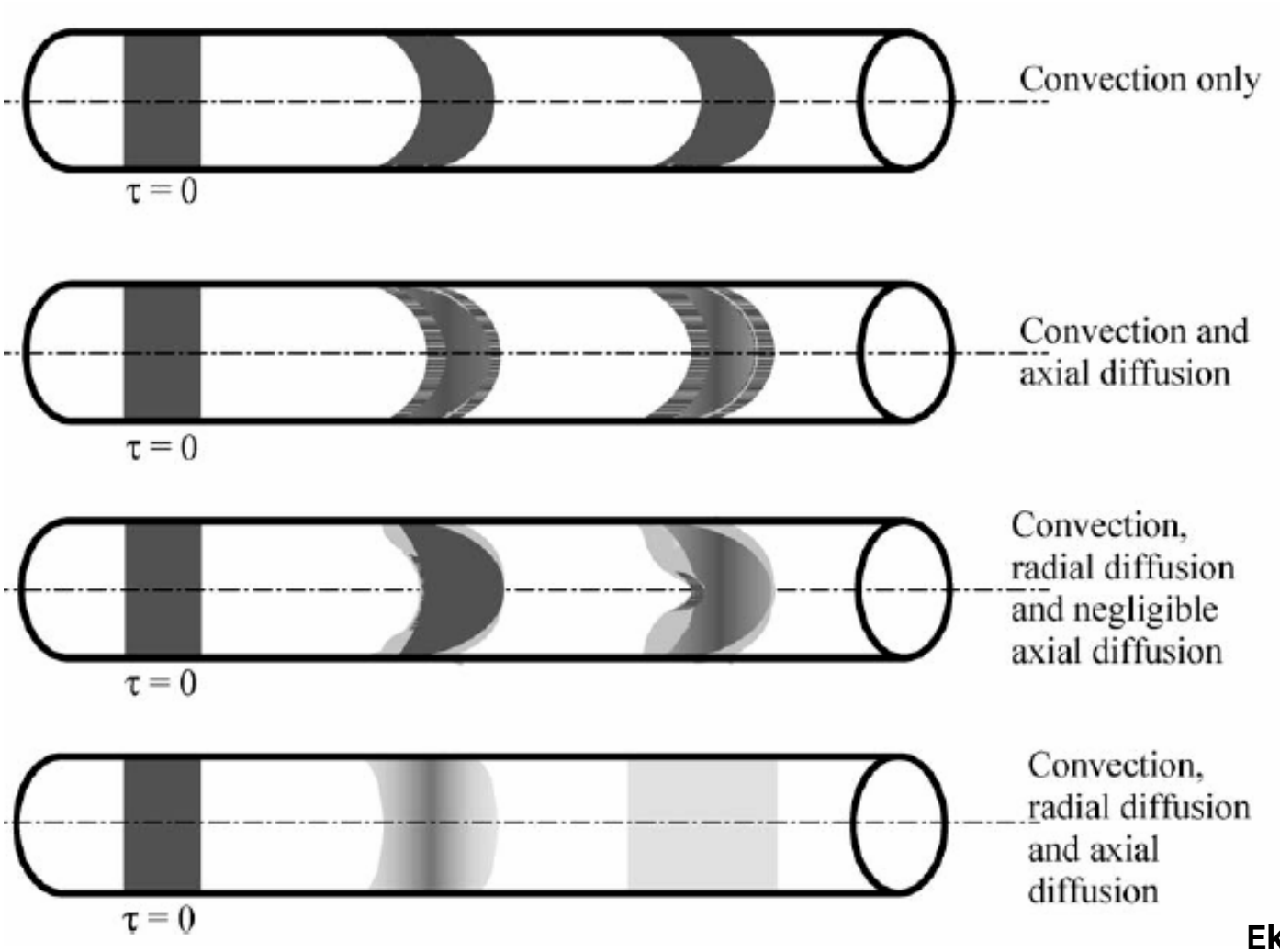
• Predicting transport phenomena of a pipe flow

- **Shanker and Lenhoff,** *AIChE* **35: 2048 (1989)**
  - Concentration profile of the tracer in a short pipe: asymmetric
  - Due to the combined effects of axial convection, radial diffusion, and axial diffusion

- **Ekambara and Joshi,** *Chem. Engin. Sci.* **59: 3929 (2004)**
  - Developed a computational model for wider ranges of:

    T (10^{-8}~10^{2}) and Pe (1-10^{5})

- **What are the axial convection, radial diffusion and axial diffusion?**



Ekambara and Joshi (2004)

• Objectives

- **Apply microfluidic system for detecting *E. coli* in laminar and turbulent pipe flows**
  - Detection limit close to 10 CFU/ml
  - Monitor *E. coli* concentrations according to time variance (real-time detection; preferably <5 min per assay)

- **Compare flow characteristics of *E. coli* and non-biological agent in a pipe**
  - Light scattering signal from microfluidic system for *E. coli*
  - Viable *E. coli* counts (not real-time)
  - Salt concentration

Materials and method

• Materials

- **Microparticles: 1 ml of 0.02% 0.92 µm highly carboxylated polystyrene particles**
- **Antibody: 1 ml of 1.023 µg ml^{-1}, anti-*E. coli* (polyclonal antibody developed in rabbit) for 33% surface coverage of antibody to microparticles**
- **70 ml of 1.5 mg ml^{-1} salt (NaCl) as non-biological tracer**

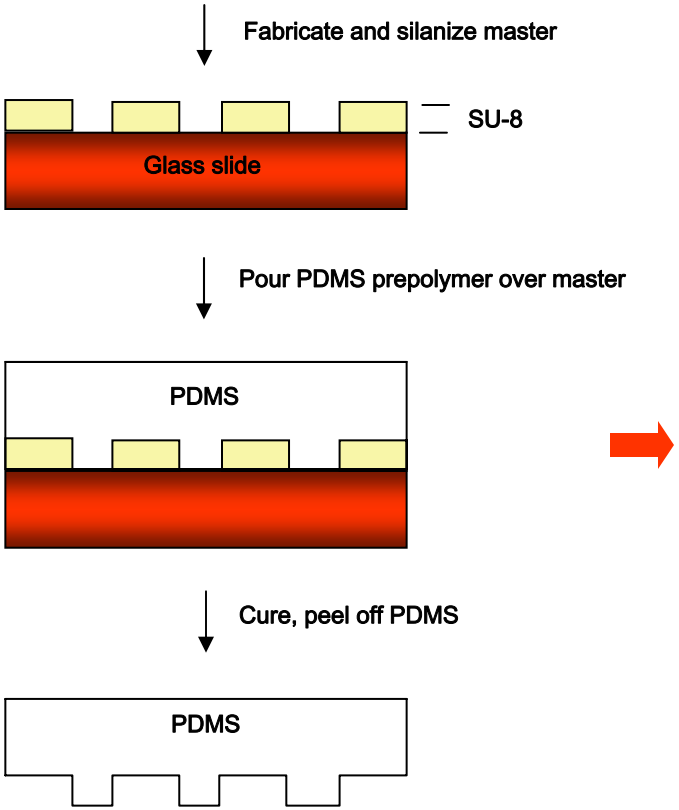
• *E. coli* assay

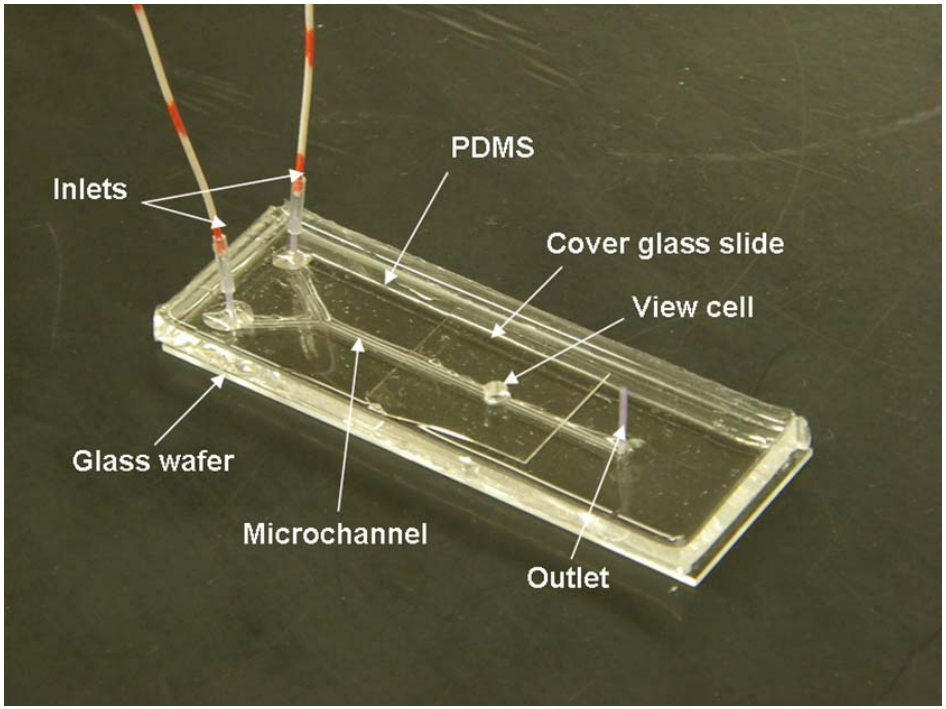
- **Target antigen: *E. coli* (ATCC 15597)**
  - **Cultured in trypticase soy broth at 37ºC for 12 hours**
  - **The strain of *E. coli* grew to stationary phase**
  - **Grown *E. coli* culture was diluted with dechlorinated tap water by 10^{-3}**

- **Counting *E. coli***
  - **Planting 0.01 ml of diluted samples to tryptic soy agar plate and incubating at 37ºC for 18 hours**
  - **Counting viable and non-viable cells: 0.2 ml of 0.1% acridine orange was added to diluted *E. coli* sample. Viable *E. coli* was confirmed through green fluorescence.**

• Fabrication of microfluidic device

- **Microfluidic devices were fabricated using standard soft lithography with PDMS molding technique**
- **The Y-junction microchannel (200 µm x 100 µm)**



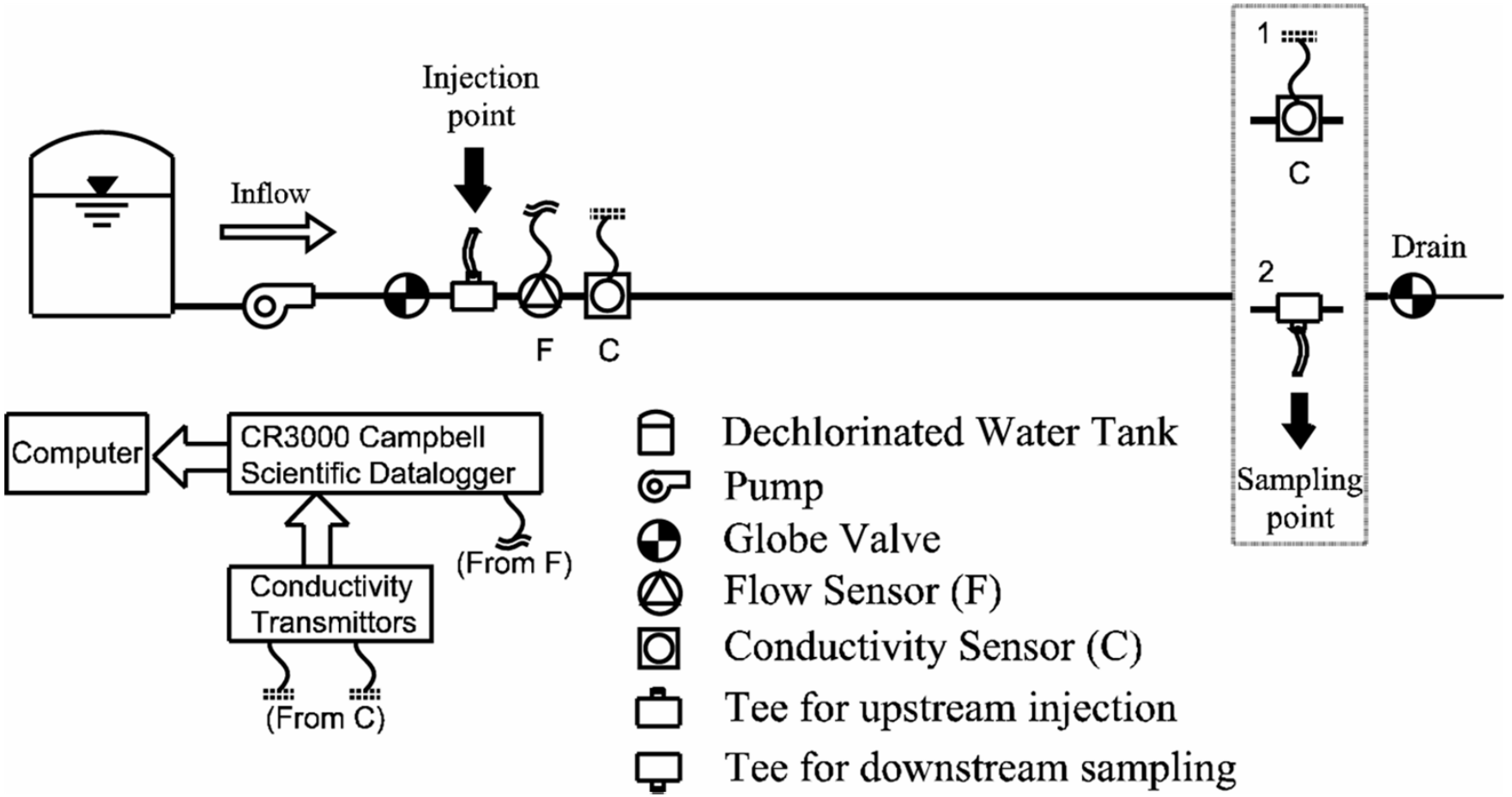


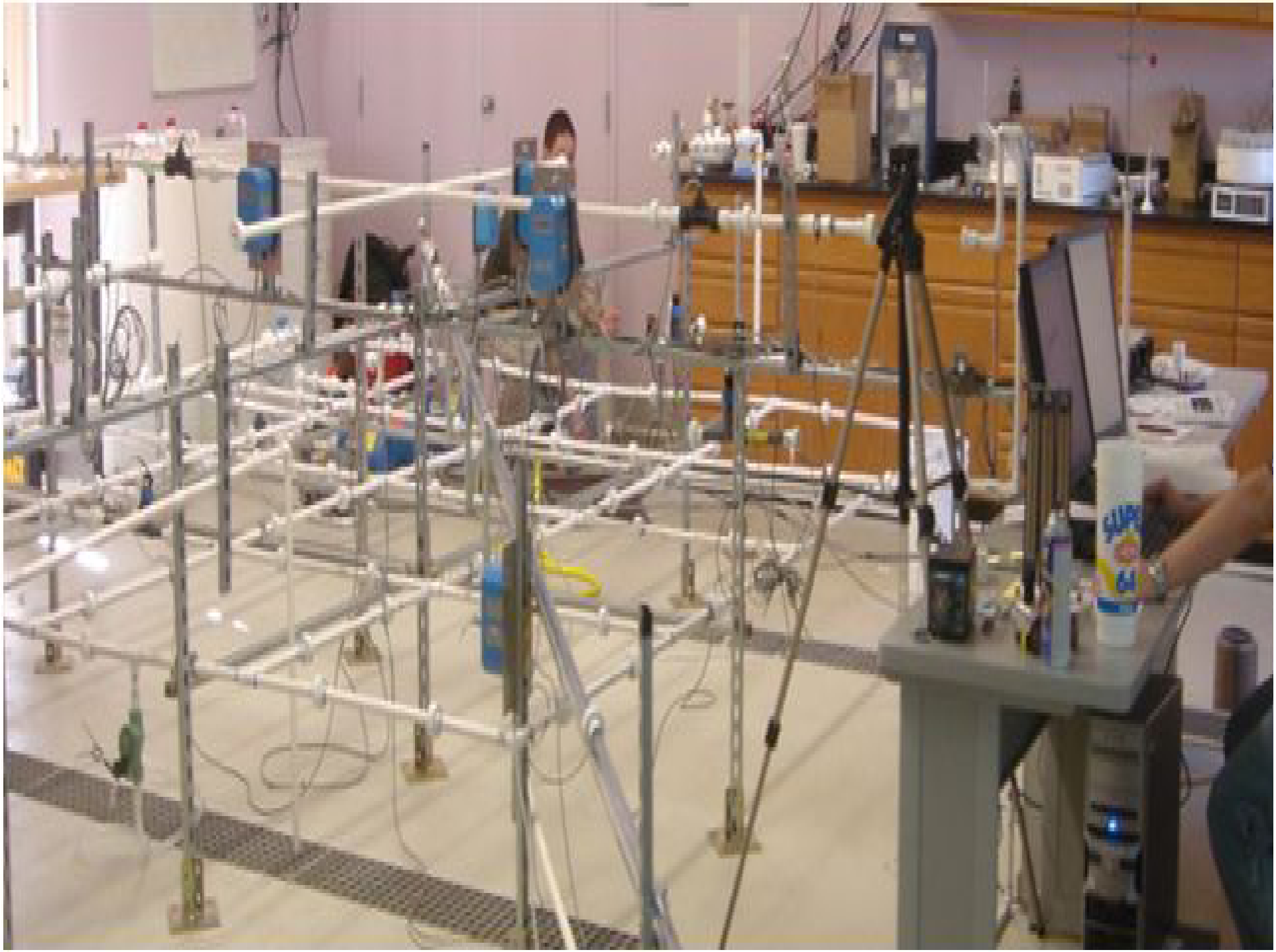
The layout of microfluidic device

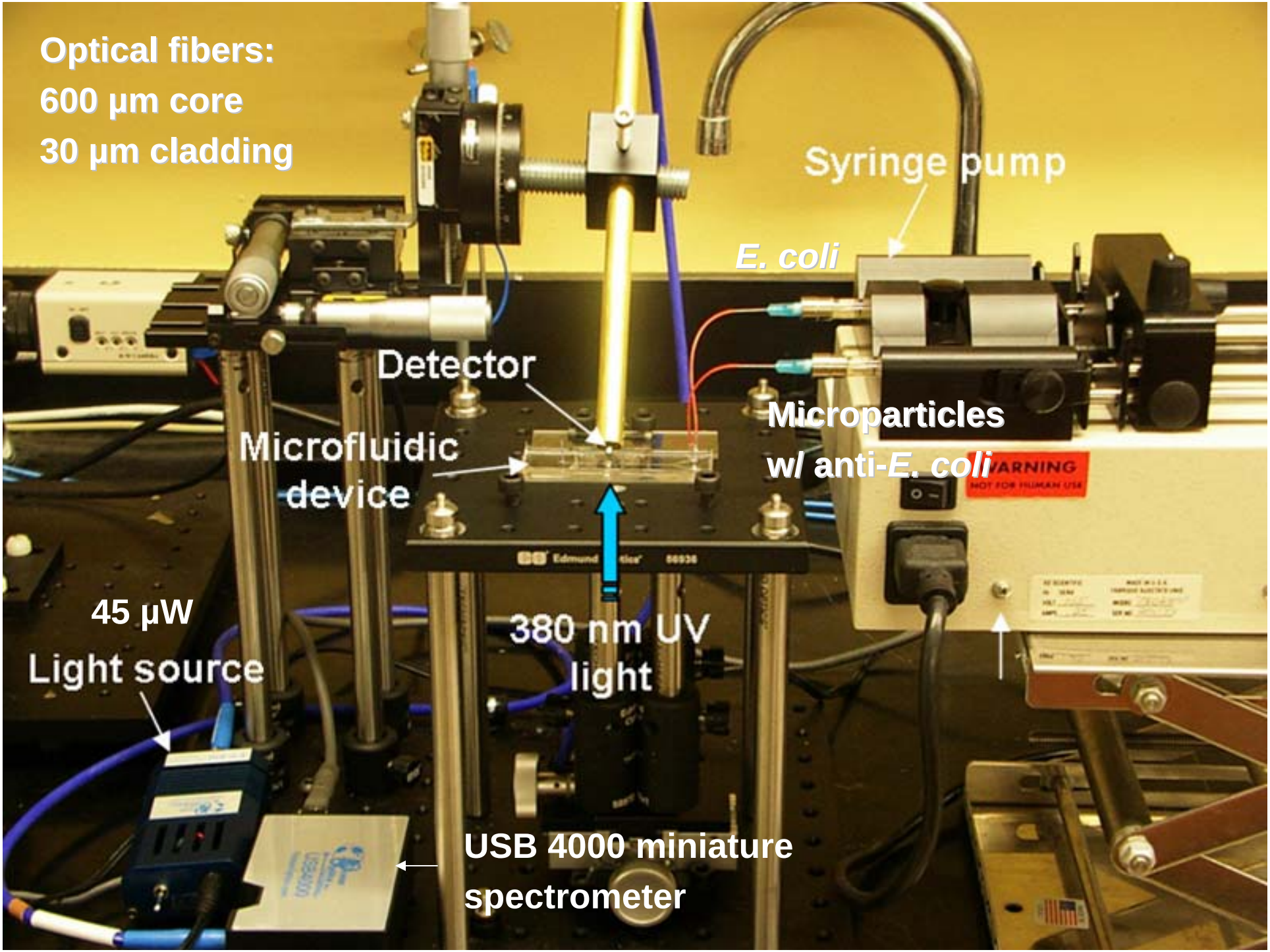
• Pipe injection experiment

- A single straight pipe: 8.5 m long, 16 mm inner diameter
- Dechlorinated tap water from a water tank: injected through the pipe system by a centrifugal pump
- Salt tracer: injected through the system at 0.4 l m^{-1} at 10 second pulse using a micro injection pump
- Flow rate: controlled by a valve at the downstream end of a pipe
- Electrical conductivity of salt: measured at both upstream and downstream ends of a pipe using conductivity meters
- The salt tracer concentration: monitored in real time using a data acquisition system
- A series of water samples were collected using a fraction collector at the downstream end of a pipe system, and applied to microfluidic system

• Model pipe system






Optical fibers:
600 µm core
30 µm cladding
Syringe pump
E. coli
Detector
Microfluidic device
Microparticles w/ anti-E. coli
45 µW Light source
380 nm UV light
USB 4000 miniature spectrometer

Results and discussion

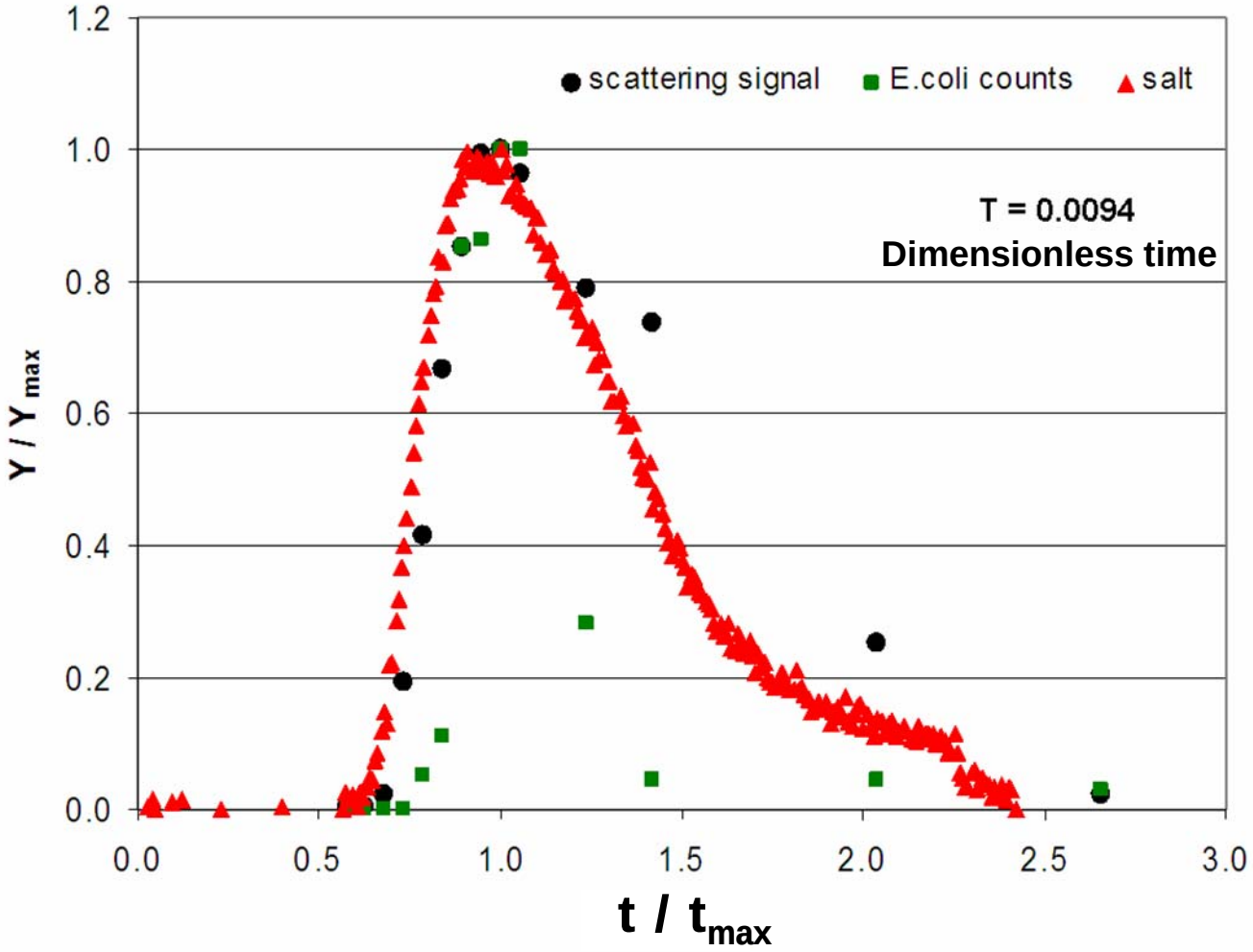
• Detection of *E. coli*

	Time (s)	E. coli (cfu ml^{-1})	Scattering		Time (s)	E. coli (cfu ml^{-1})	Scattering
Laminar	Blank		1217±2	Turbulent	Blank		1253±8
	65	10	1225±4*		17	0	1257±18
	71	10	1232±4*		19	0	1271±5
	77	30	1277±2*		21	48355	1540±15*
	83	12000	1743±37*		23	987331	1669±27*
	89	26000	2350±43*		25	12630566	1939±45*
	95	197000	3044±39*		27	9983316	2102±86*
	101	200000	3541±18*		29	3120125	1607±80*
	107	231000	3930±48*		31	225257	1388±45*
	113	231279	3950±66*		33	58217	1343±35*
	119	64877	3853±66*		36	8509	1313±7*
	140	10697	3376±142*		42	985	1252±33*
	160	10221	3232±22*				
	230	6825	1908±20*				
	300	2985	1282±6*				
RSD (%)	0-4			RSD (%)	0-2		

- **Detection range**
  - 10-10^6 cfu ml^{-1} (laminar), 10^2-10^7 cfu ml^{-1} (turbulent)
  - Low detection limit: 10 cfu ml^{-1} (includes not only viable cells but also dead cell and free antigens)

- **Detects not just viable *E. coli*, but also dead cells and free antigens**
  - *E. coli* counts = viable *E. coli* only
  - Light scattering signal = viable cells + non-viable cells + free antigens = total *E. coli (Han et al., Biosens. Bioelectron.* **doi:10.1016/j.bios.2007.11.013, 2008)**

• Axial dispersion of *E. coli* in laminar flow

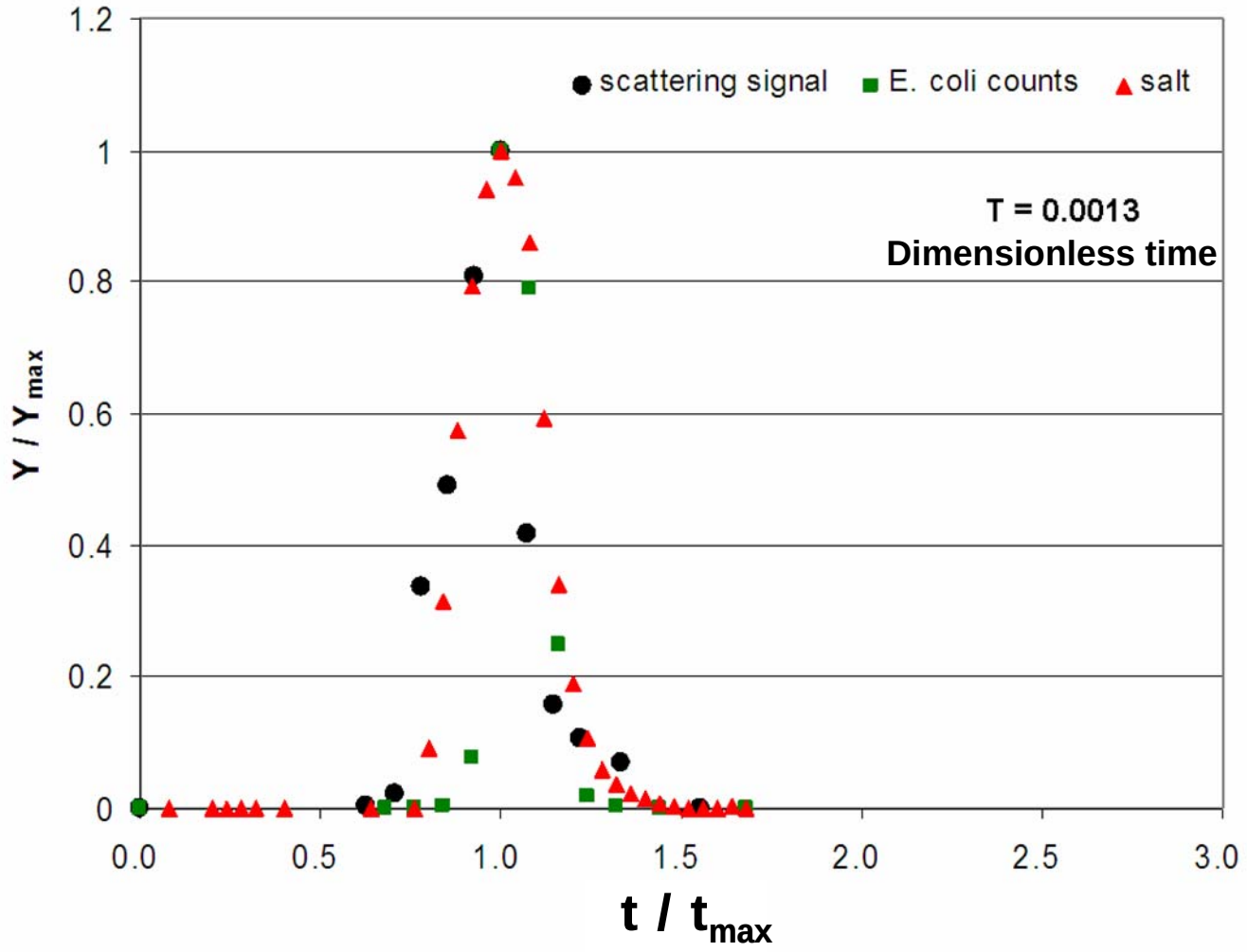


• Axial dispersion of *E. coli* in laminar flow

- **Total *E. coli* and salt showed almost the same concentration profile (asymmetric)**
  - More likely axial convection and axial/radial diffusion, rather than self-motility of *E. coli* or *E. coli* fouling to a pipe wall

- **Viable *E. coli* profile was symmetric**
  - Asymmetry originates from non-viable cells and free antigens (i.e. cell fragments)
  - Axial convection and axial/radial diffusion are the issues of cell fragments, not viable cells

• Axial dispersion of *E. coli* in turbulent flow



• Detection range and viability issue

- **All three curves showed symmetric profiles**
  - Self-motility of *E. coli* do not contribute to the concentration profile
  - *E. coli* fouling to a pipe wall is also negligible

• Conclusion

- *E. coli* **could be detected down to 10 cfu ml^{-1} level in real time**
- **Cell fragments contributed to axial convection, radial/axial diffusion in laminar flow, just like salts**
- **Viable cells do not have axial diffusion and radial diffusion issue in laminar flow**
- **No axial convection, radial/axial diffusion in turbulent flow, for both salt and** *E. coli*

• Acknowledgments

- Micro/Nano fabrication center at the University of Arizona for the cleanroom facility and subsequent equipment assistance
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